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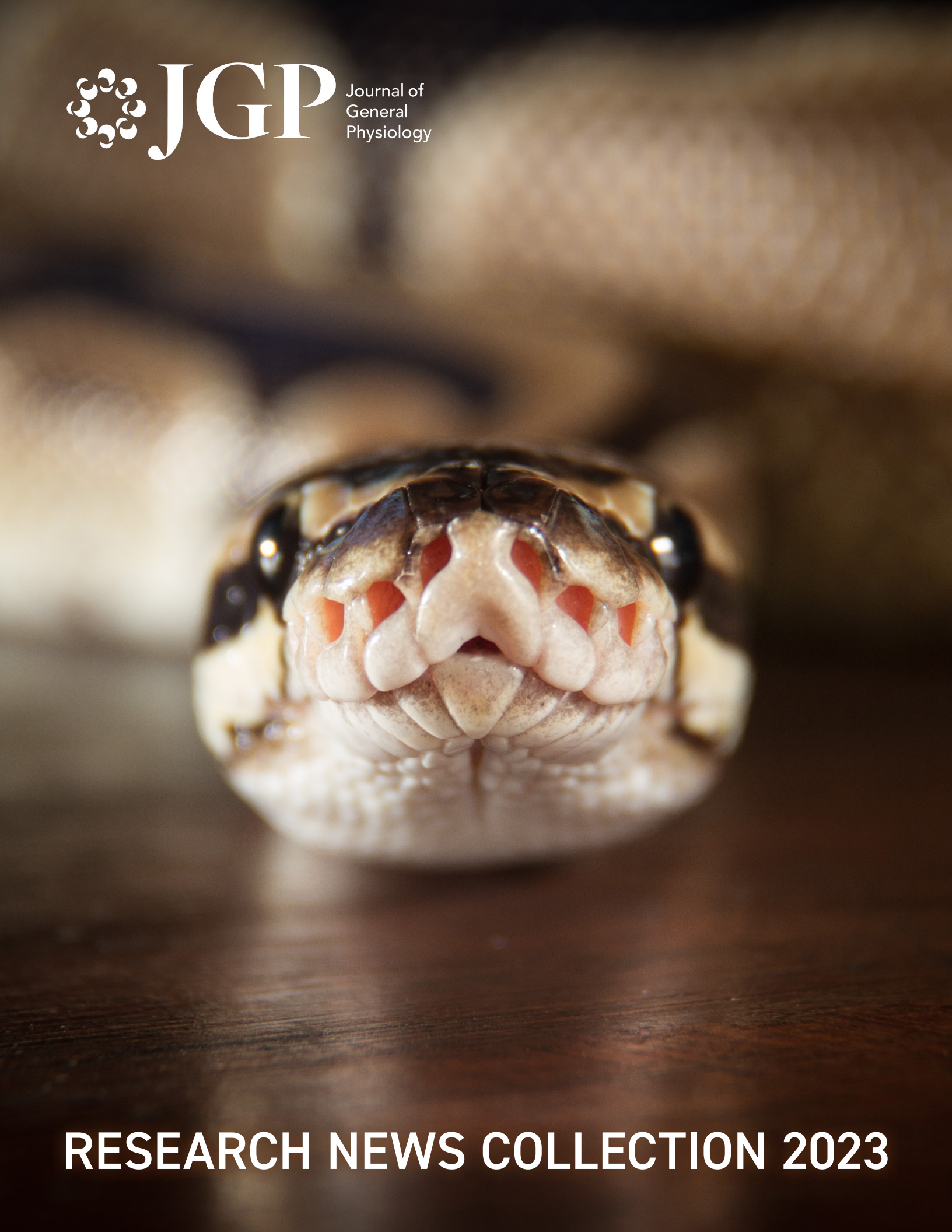

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On the cover: A stock image of a *Python regius*, Credit: iStock.com/RichardCardenas. Learn about the research Boukens et al. performed with ball pythons in "Catecholamines help snakes have a change of heart" on page 5.

On the table of contents: A stock image of *Centruroides margaritatus*, Credit: iStock.com/Clinton Harris. Learn more about the scorpion toxin research performed by Naseem et al. on page 11.

JGP RESEARCH NEWS 2023

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Catecholamines help snakes have a change of heart

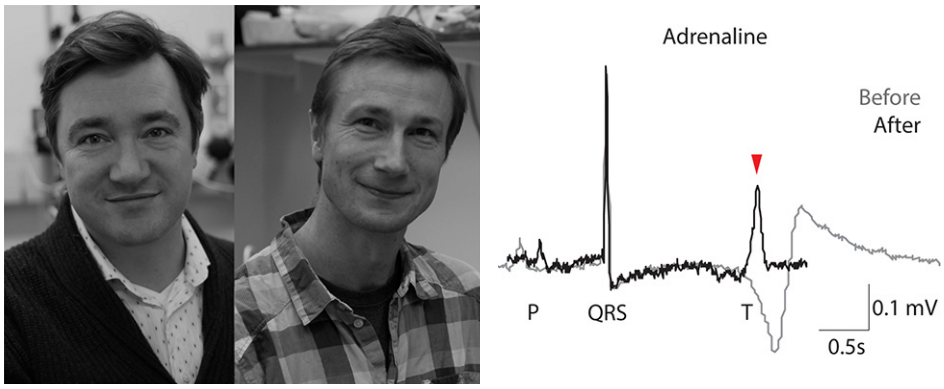
Study on python snakes reveals that the regulation of ventricular repolarization by the sympathetic nervous system is evolutionarily conserved

The T-wave of an electrocardiogram (ECG) arises from local differences in ventricular repolarization and represents a vulnerable period for the generation of arrythmias when some, but not all, of the myocardium is still refractory and unable to generate a new action potential. In mammals, ventricular repolarization is regulated by catecholamines released by the autonomic nervous system. Boukens et al. show that this mode of regulation is conserved in the ball python, *Python regius* (1).

Working together at Amsterdam UMC, Bas Boukens and Bjarke Jensen are interested in the electrophysiological adaptations that have occurred during cardiac evolution. Coldblooded reptiles have a much longer ventricular repolarization phase than warm-blooded mammals, even at 37°C (2). Moreover, the T-wave is typically negative in reptiles, whereas in mammals it is usually positive. Anecdotal observations, however, suggest that, in some reptiles, the T-wave can invert and become positive at higher body temperatures (3, 4). "We were curious about what might underlie these observations," Jensen says.

The researchers therefore recorded ECGs in living ball pythons as their body temperatures were increased (1). The ball python's heart is unique in having functionally distinct ventricles, with a high-pressure left side and a low-pressure right side, even though, as in other snakes, the two sides are not anatomically separated. Though results varied across individual pythons and ECG leads, raising body temperature from 25 to 35°C caused an inversion of the snakes' T-wave, reflecting temperature-dependent changes in the pattern of ventricular repolarization.

In 1880, Burdon-Sanderson and Page (5) showed in their classic experiments that local differences in temperature change T-wave polarity in the ectothermic heart, presumably due to a direct effect of temperature on the activity of cardiac ion channels. However, when Boukens et al. recorded an ECG from a decapitated python, they found that raising temperature did not cause T-wave inversion (1). "So, we realized that it might not be a direct effect of temperature



Bas Boukens (left), Bjarke Jensen (center), and colleagues reveal that, similar to mammals, catecholamines released by the autonomic nervous system regulate ventricular repolarization in ball pythons. An ECG (right) shows that, by altering the pattern of ventricular repolarization, adrenaline treatment causes an inversion of the T-wave (red arrowhead). A similar phenomenon is observed in snakes undergoing a rise in body temperature, when autonomic tone increases.

but might involve another factor, namely catecholamines released by the autonomic nervous system," says Boukens. Autonomic activity increases at higher temperatures, but the ability of catecholamines to modulate ventricular repolarization would be blunted in decapitated snakes lacking a functional nervous system.

Sure enough, the researchers found that stimulating the β-adrenergic receptor induced T-wave inversion in pythons maintained at a stable temperature. In contrast, the β blocker propranolol largely prevented higher temperatures from inducing T-wave inversion.

Thus, similar to mammals, catecholamines regulate ventricular repolarization in ball pythons, and the increase in autonomic tone at higher temperatures alters the pattern of repolarization and changes T-wave shape. "The T-wave inversion suggests that certain regions of the python heart respond more strongly to adrenergic stimulation than other regions," Boukens says.

To test this idea, the researchers performed RNA sequencing of tissue samples taken from different regions of the python heart. "Catecholamine-associated genes exhibited differential expression between the left and right sides of the ventricle, consistent with the repolarization of these regions being differentially modulated by adrenergic signaling," says Jensen.

This may provide some sort of advantage to pythons as their body temperature rises, though the resulting changes in repolarization pattern could also leave them vulnerable to developing arrythmias. Boukens and Jensen are now extending their studies to a different branch of the evolutionary tree, examining repolarization and arrhythmogenesis in zebra finches (6).

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JGP Journal of General Physiology

Troponin levels make a difference

Lower troponin expression in the right ventricle underlies interventricular differences in excitation–contraction coupling

The right ventricle of the heart pumps blood into the pulmonary circulation, and therefore experiences lower afterload pressure than the left ventricle, which pushes blood around the entire rest of the body. This functional difference is reflected not only at the anatomical level—the right ventricular wall is thinner than the left—but also at the cellular level, where cardiomyocytes in the left and right ventricles are thought to demonstrate differences in excitation– contraction (E–C) coupling. Jeon et al. carefully characterize these interventricular differences and show that they arise, in part, from lower troponin expression in the right ventricle (1).

Young Keul Jeon, Jae Won Kwon, Yin Hua Zhang, Sung Joon Kim (left to right), and colleagues reveal that E–C coupling differs between the right and left ventricles, partly because troponin complex expression is lower in RVCMs. For example, even though action potential duration is shorter in RVCMs, Ca^{2+} transients have a similar amplitude in both LVCMs and RVCMs, while the transient decay rate is slower in RVCMs.

E–C coupling is the process by which an action potential triggers a transient rise in cytosolic Ca^{2+} that initiates myofibril sliding and cardiomyocyte contraction. Previous studies have shown that action potential duration is shorter in right ventricular cardiomyocytes (RVCM) than in left ventricular cells (LVCM; 2, 3). "However, experimental findings on the differences in Ca^{2+} transients and sarcomere shortening kinetics are not consistent and demand a more rigorous approach," explains Sung Joon Kim, a professor at Seoul National University College of Medicine. Kim and colleagues, including co-first authors Young Keul Jeon and Jae Won Kwon, and co-corresponding author Yin Hua Zhang, isolated cardiomyocytes from the left and right ventricles of rats and subjected them to a variety of assays (1). Whole-cell patch clamping confirmed that action potential duration is shorter in RVCMs and that this is largely because a higher density of transient outward K^+ current drives faster repolarization. This, in turn, reduces the influx of Ca^{2+} into RVCMs.

"Surprisingly, despite the shorter action potential duration in RVCM, the amplitude of the Ca^{2+} transient was not different, while the decay of the Ca^{2+} transient was slower in RVCM than LVCM," Kim says.

The slower decay of cytosolic Ca^{2+} levels in RVCMs is mainly due to a 60% reduction in the activity of the SERCA ATPase that pumps Ca^{2+} back into the SR. But why do cytosolic Ca^{2+} levels show similar peaks in RVCMs and LVCMs? Kim and colleagues found that, compared with LVCMs, RVCMs express lower levels of the cardiac troponin complex that regulates the interaction between actin and myosin filaments in the sarcomere. Decreased expression of the Ca^{2+}- binding troponin subunit TnC appears to reduce the Ca^{2+} buffering capacity of RVCMs, allowing cytosolic Ca^{2+} levels to reach similar peaks to those seen in LVCMs, despite the interventricular differences in action potential duration.

However, though Ca^{2+} transients show similar amplitudes in RVCMs and LVCMs, Kim and colleagues found that contractility—as measured by changes in sarcomere length—is reduced in RVCMs. "We suggest that this phenomenon might be caused by the lower expression of the troponin complex," Kim says.

Indeed, when the researchers introduced the specific features of RVCMs—higher transient outward K^+ current, lower SERCA activity, and reduced troponin expression—into computational models previously developed for LVCMs (4, 5), they were able to recapitulate all of their experimental measurements obtained from RVCMs, including the shorter action potential duration, the amplitude and decay rate of the Ca^{2+} transient, and the reduction in contractility.

Lower troponin expression therefore significantly alters E–C coupling in RVCMs. Kim and colleagues note that the reduced Ca^{2+} buffering capacity of RVCMs may make the right ventricle more susceptible to arrhythmias, something they now plan to investigate using an animal model of pulmonary arterial hypertension and right ventricular failure.

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Gap junctions and hemichannels keep the RPE connected

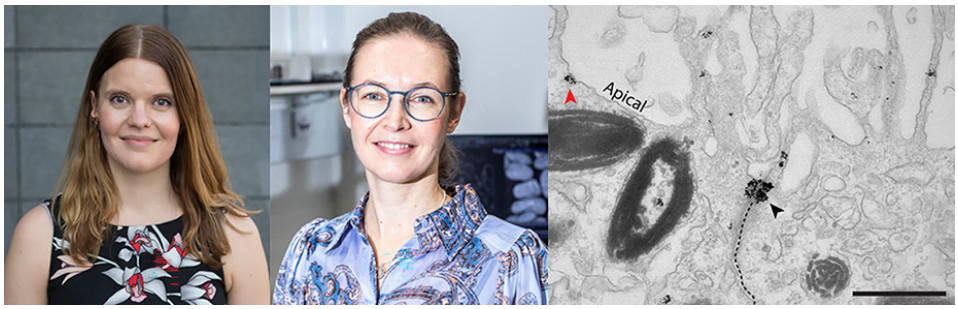
The electrical properties of the retinal pigment epithelium are influenced by connexin-based gap junctions and hemichannels

The retinal pigment epithelium (RPE) is a layer of densely pigmented cells that underlies the retina and supports the function of photoreceptors. Some of these support functions—such as the secretion of growth factors and the renewal of photoreceptor membranes via phagocytosis of their outer segments—are regulated by voltage-gated ion channels (1, 2), suggesting that the electrical properties of the RPE are important for retinal health and disease. Fadjukov et al. reveal that these electrical properties are regulated by both gap junctions and connexin hemichannels in the RPE (3).

Gap junctions are composed of connexin proteins, which form hexameric hemichannels in the membranes of many cell types. Hemichannels in neighboring cells can align to create a gap junction that allows small molecules and ions to pass between cells, but individual hemichannels can have gap junction–independent functions as well.

Gap junctions are particularly prominent in the RPE, where they could facilitate electrical coupling between cells and potentially help synchronize the RPE's functions across the epithelial monolayer. Studies in amphibians revealed that RPE cells are, indeed, highly electrically coupled to each other (4); however, the connectivity of the mammalian RPE, and the contribution of gap junctions to the tissue's electrical properties, are largely unknown. Soile Nymark and colleagues at Tampere University, Finland, recently developed the ability to perform patch clamp recordings of single RPE cells in intact monolayers (1). "So, we wanted to look more closely at the electrical characteristics of the mammalian RPE," Nymark says.

Electrical conductance through gap junctions is likely to lower a cell's input resistance, thereby reducing its electrical excitability. Nymark and colleagues, including first author Julia Fadjukov, measured the input resistance of RPE cells in monolayer cultures derived from human embryonic stem cells and also in intact RPE isolated from mouse eyes (3). In both cases, treating cells with the gap junction blocking agent meclofenamic acid (MFA) dramatically increased input resistance, and this effect was reversed when the drug was washed out.



Julia Fadjukov (left), Soile Nymark (center), and colleagues reveal that gap junctions and connexin hemichannels influence the electrical properties of the mammalian retinal pigment epithelium. Immuno- EM of human embryonic stem cell–derived RPE (right) shows the presence of connexin 43 in both gap junctions (black arrowhead) and apical hemichannels (red arrowhead). Credit: Author photos by Miika Fadjukov, Fadjukov Photoshooting and Jonne Renvall, Tampere University.

The researchers then switched to a dual patch clamp configuration to measure the extent of electrical coupling between cells. Neighboring RPE cells were, indeed, highly coupled, and this connectivity was abolished by the addition of MFA. Coupling between non-adjacent cells was significantly smaller, however, suggesting that, at least under baseline conditions, the connectivity of mammalian RPE monolayers is relatively low. "However, we think this can be dynamically regulated to allow fast spreading of ions and other signaling molecules across the epithelium," Nymark says.

Based on their measurements, Nymark and colleagues worked with Sophia Wienbar and Gregory Schwartz at Northwestern University to construct a computational model of an RPE cell network. The model revealed that inhibiting gap junctions cannot be the only mechanism by which MFA increases input resistance. Indeed, MFA also blocks individual hemichannels, and Fadjukov et al. determined that there are functional hemichannels in the apical membrane of RPE cells.

To test whether these hemichannels contribute to input resistance, the researchersused TAT-Gap19, a specific inhibitor of hemichannels formed by connexin 43, the most prominent connexin in RPE cells. Sure enough, TAT-Gap19 treatment increased input resistance, indicating that hemichannels, as well as gap junctions, influence the electrical properties of the RPE.

Nymark and colleagues now want to investigate whether the connectivity provided by gap junctions helps the physiological processes in the RPE that require precise synchronization, as well as the intriguing possibility that the apical hemichannels facilitate signaling between the RPE and the retina.

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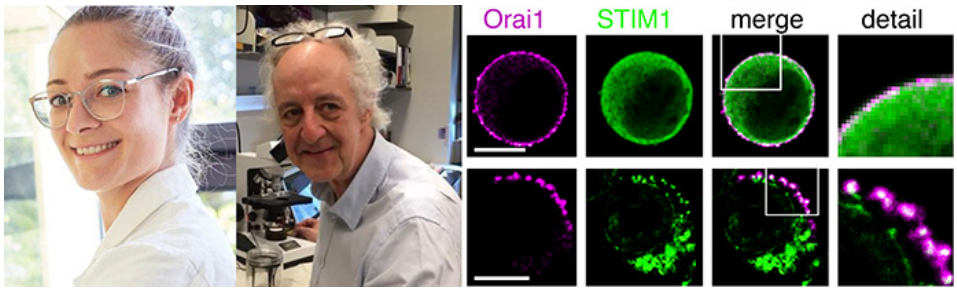
X rays activate T cell calcium signaling

Clinically relevant doses of ionizing radiation induce an immune response in T cells by triggering the store-operated Ca^{2+} entry pathway

Ionizing radiation (IR) is a key anti-cancer treatment due to its ability to induce DNA damage and cell death. But the effects of IR on other cellular pathways, in both tumors and neighboring healthy tissues, is much less understood. Tandl et al. show that x rays trigger an immune response in T cells by activating the store-operated Ca^{2+} entry (SOCE) pathway, a finding that could have important implications for both the toxic and therapeutic effects of radiotherapy (1).



Dominique Tandl (left), Gerhard Thiel (center), and colleagues reveal that x-ray irradiation activates T cells by stimulating the SOCE pathway. Compared to unirradiated control cells (top row), clinically relevant x-ray doses (bottom row) cause Orai1 (magenta) and STIM1 (green) to cluster at ER–plasma membrane contact sites where they mediate Ca^{2+} influx. This leads to cytosolic Ca^{2+} oscillations and nuclear translocation of the key T cell transcription factor NFAT.

Irradiation of patient tumors inevitably impacts blood cells circulating through the targeted tissue. While some studies have reported immunosuppressive effects of irradiation, others have shown that IR can stimulate the immune system. Indeed, combining radiotherapy with immune checkpoint inhibitors has synergistic impacts on tumor growth (2, 3). Gerhard Thiel and colleagues at Technische Univesita¨t Darmstadt recently found that clinically relevant x-ray doses activate T lymphocytes (4). "We found that irradiated T cells become bigger and start to adhere, which are indicators of an active immune response, and that this was likely associated with changes in Ca^{2+} signaling," Thiel says.

To investigate these changes, Thiel and colleagues, including graduate student Dominique Tandl, loaded Jurkat cells (a leukemic T-cell line) with a Ca^{2+}-sensitive dye and imaged them in real time with a fluorescent microscope directly coupled to an x-ray source (1). Clinically relevant x-ray doses had no immediate impact on the cytosolic Ca^{2+} concentration of T cells. After a delay of ~10–70 min, however, the majority of irradiated T cells began to show rapid oscillations in their cytosolic Ca^{2+} levels. These oscillations were suppressed by the removal of extracellular Ca^{2+} or the addition of a broad spectrum Ca^{2+} channel blocker, indicating that they depend on the influx of Ca^{2+} into the cell.

The major mechanism of Ca^{2+} entry into T cells is the SOCE pathway (5), in which the depletion of Ca^{2+} stores in the ER triggers the clustering of STIM1 and Orai1 proteins at ER– plasma membrane contact sites, where they form calcium release–activated calcium (CRAC) channels that mediate Ca^{2+} influx. Tandl et al. found that x-ray irradiation triggers STIM1/Orai1 clustering in T cells. Crucially, treating T cells with a CRAC channel inhibitor, or knocking out the Orai1 protein, reduced x-ray–induced Ca^{2+} oscillations.

The researchers also found that irradiation initiates the SOCE pathway by depleting Ca^{2+} levels in the ER. The delayed onset of Ca^{2+} oscillations suggests that this isn't because x rays disrupt the integrity of the ER membrane. Instead, the researchers speculate, the reactive oxygen species generated by irradiation may modulate the activity of ER Ca^{2+} channels or pumps.

In response to T cell receptor activation, SOCE- mediated Ca^{2+} oscillations stimulate translocation of the transcription factor NFAT into the nucleus, where it induces the expression of numerous genes crucial for T cell immune function. Tandl et al. found that x-ray irradiation also induces NFAT's movement into the nucleus, and that blocking Ca^{2+} oscillations with a CRAC channel inhibitor prevents this translocation, as well as the subsequent increase in cell size associated with T cell activation. Taken together, Tandl et al.'s findings reveal how x-rays can stimulate the immune function of T cells. In the short term, Thiel plans to investigate how IR depletes ER Ca^{2+} stores. In the longer term, however, he hopes that their studies will lead to improvements in cancer therapy. "It may be possible to optimize the killing effect of radiation on tumor cells, while also inducing the positive effect of immune stimulation," Thiel says.

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A Kv2 inhibitor traps itself in place

Voltage activation, but not channel opening, is required for RY785 to access the central cavity of Kv2 channels, where it promotes voltage sensor deactivation to trap itself in place

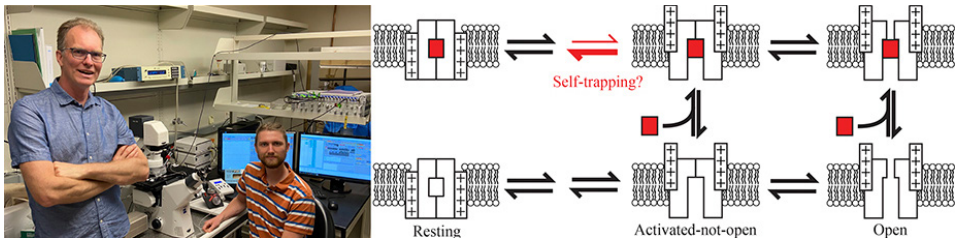
The Kv2 family of voltage-gated K^+ channels are expressed in a wide variety of cell types, including neurons, where they modulate repetitive action potential firing (1), and pancreatic β cells, where they suppress insulin secretion (2). Marquis and Sack investigate how a drug called RY785 inhibits Kv2 channels, providing potential new insights into the mechanism of Kv2 channel gating (3).

Ion channel inhibitors are useful tools for elucidating channel function, but understanding the precise mechanism of an inhibitor's action is crucial for interpreting its physiological effects. The small molecule RY785 is a potent and selective inhibitor of Kv2 channels that shows a use-dependent onset of inhibition, i.e., voltage activation of the channel is required for the drug to begin inhibiting the K^+ current (4). "We wanted to figure out how this drug works," says Jon Sack, an associate professor at the University of California, Davis.

Sack, along with graduate student Matthew Marquis, expressed rat Kv2.1 channels in CHO-K1 cells and found that 1 µM RY785 completely inhibited channel conductance at all physiologically relevant voltages (3). As expected, voltage activation of rat Kv2.1 channels was required for the onset of RY785 inhibition, but Marquis and Sack found that recovery from inhibition is also voltage dependent: voltage activation of Kv2.1 channels speeds the restoration of K^+ currents after RY785 washout, whereas channels held at negative membrane potentials barely recover any conductance at all.

The simplest explanation for these observations is that voltage activation induces a conformational change in Kv2 channels that allows RY785 to access its binding site within the channel. The drug may then become trapped at this binding site when the channel returns to its resting, deactivated conformation, and can only be released if the channel is voltage activated once more.

To learn more about the conformational changes associated with RY785 binding, Marquis and Sack analyzed Kv2.1 gating currents in the presence and absence of the drug. Remarkably, they found that RY785 accelerates the movements associated with channel deactivation. "So, it looks like a voltage-activated gate has to open for RY785 to access the channel, but then the drug pulls the gate closed behind it," Sack says. This "self-trapping" mechanism would explain RY785's high affinity for and slow off rate from Kv2 channels.



Matthew Marquis (seated) and Jon Sack (standing) determine the mechanism of use-dependence of RY785, an inhibitor of Kv2 channels. Their proposed model (right) suggests that the drug (red rectangle) can access the central cavity of Kv2 channels when they are in an activated-not-open conformation. The drug then promotes channel deactivation, trapping itself in place.

But where in the channel does RY785 bind? Marquis and Sack found that RY785 competes with the classic open-channel blocker tetraethylammonium, which binds to a site in the central cavity of voltage-gated K^+ channels. Surprisingly, however, the researchers also determined that channel opening is not required for the onset of RY785 inhibition. Thus, Kv2 channels adopt an "activated-not-open" conformation, in which, they speculate, the central cavity is accessible from the cytosol (allowing drugs such as RY785 to enter and bind), but the channel is not open to the passage of K^+ ions.

This suggests that, unlike Kv1 channels in which opening of an intracellular gate seems to be the final activation step (5), Kv2 channel opening also requires opening of a second gate. This might explain why Kv2 channels open more slowly than other voltage-gated K^+ channels and are more involved in the modulation of repetitive firing than of isolated action potentials.

"The use of two activation gates could be important for the physiological role of Kv2 channels," Sack suggests. "Now we want to know what these two gates really are and how they work together."

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JGP Journal of General Physiology

How a tyrosine primes the pump

Study uses both natural and unnatural amino acid substitutions to examine how a key tyrosine residue controls the selectivity of the Na^+/K^+ pump

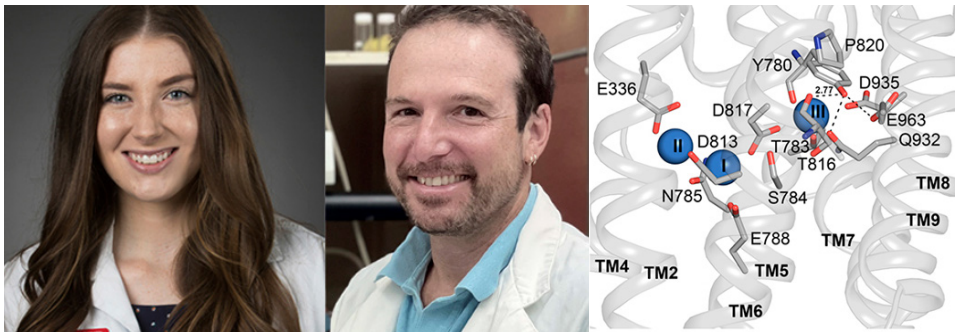
Numerous cellular processes, including nutrient uptake and the transmission of nerve impulses, depend on the electrochemical gradients of Na^+ and K^+ ions across the plasma membrane. These gradients are established and maintained by the Na^+/K^+ pump, a P-type ATPase that expels three Na^+ ions and imports two K^+ ions with every round of ATP hydrolysis. Spontarelli et al. (1) probe the function of a critical tyrosine residue in the Na^+/K^+ pump's ion selectivity.

The Na^+/K^+ pump has three cation-binding sites. Sites I and II somehow change their selectivity depending on the pump's conformation, binding Na^+ at the intracellular side of the membrane and K^+ at the extracellular side. Site III, in contrast, specifically binds Na^+ and contains a critical tyrosine residue that is well conserved across all P-type ATPases, including the neuron-specific Na^+/K^+ pump ATP1A3, where its mutation is linked to the neurological disorder alternating hemiplegia of childhood (2).

The crystal structure of the Na^+/K^+ pump shows that the side chain of this tyrosine residue sits immediately above the bound Na^+ ion at site III, suggesting that the phenol ring's π electrons could directly contribute to cation binding (3). However, the tyrosine residue could also contribute indirectly by participating in a hydrogen-bond network with other amino acids that coordinate the Na^+ ion. "We wanted to investigate how this conserved tyrosine residue contributes to ion binding," explains Pablo Artigas from Texas Tech University Health Sciences Center.

Artigas and colleagues, led by first author Kerri Spontarelli, generated versions of the *Xenopus laevis* Na^+/K^+ pump in which this critical tyrosine residue—Y780—was mutated to other amino acids (1). They expressed these proteins in *Xenopus* oocytes or COS-1 cells and determined their affinities for cations using electrophysiology and, with the help of Bente Vilsen's group at Aarhus University, biochemical assays.

Replacing Y780 with phenylalanine—maintaining the aromatic ring and its π electrons but removing the hydroxyl group capable of forming H-bonds—dramatically



Kerri Spontarelli (left), Pablo Artigas (center), and colleagues investigate the role of a conserved tyrosine residue in the ion selectivity of the Na^+/K^+ pump, using non-sense suppression to substitute this residue with unnatural, fluorinated analogs. The study suggests that the tyrosine (Y780 in the *Xenopus* pump) mainly contributes to Na^+ binding at site III by forming H-bonds with neighboring residues, rather than through a cation–π interaction.

reduced the pump's affinity for Na^+ at both the internal and external sides of the membrane. Other substitutions that disrupt the H-bond network also reduced the apparent affinity for Na^+, whereas mutating Y780 to glutamine increased the pump's affinity for Na^+, probably by enabling the formation of additional H-bonds between site III residues.

This suggests that the H-bonds formed by Y780 are more important than the cation–π interaction for Na^+ binding. To address this question more precisely, Spontarelli et al. worked with Christopher Ahern's lab at the University of Iowa to introduce unnatural, fluorinated derivatives of tyrosine or phenylalanine at position 780 using non-sense suppression. The fluoro group withdraws electrons from the aromatic ring, weakening any potential cation–π interaction. The technique has previously been used to study the roles of aromatic residues in ion channels (4), but is more challenging for pumps, whose lower transport rates necessitate much higher levels of protein expression.

Nevertheless, by repeatedly injecting cells with tRNAs carrying the fluorinated amino acids, Spontarelli et al. were able to express sufficient amounts of the mutant pumps to estimate the change in affinity for Na^+ induced by the substitutions. "We observed that fluorination has very little effect on Na^+ binding," Artigas says, indicating a minimal contribution of cation–π interaction to Na^+ coordination.

Instead, it appears that the H-bond network formed by Y780 is critical for Na^+ binding at site III. Intriguingly, Spontarelli et al. found that mutating Y780 also reduced the Na^+/K^+ pump's apparent affinity for K^+, suggesting that it participates in a complex H-bond network proposed to control the cation selectivity switch at sites I and II as the pump changes its conformation (5). Artigas and colleagues now plan to extend their use of unnatural amino acids to understand this selectivity switch in more detail.

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A scorpion toxin takes the sting out of T cell activation

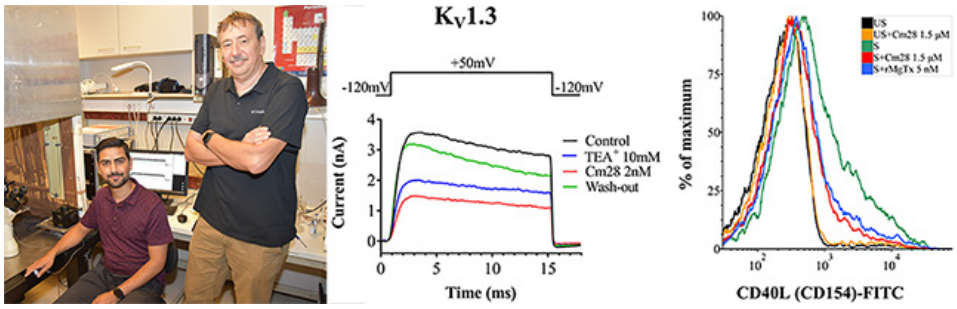
A novel peptide in scorpion venom that inhibits $K_V1.2$ and $K_V1.3$ channels could form the basis for new treatments for autoimmune diseases

Scorpions produce hundreds of biomolecules, many of which target voltage-gated potassium (K_V) channels. Naseem et al. (1) characterize a novel scorpion toxin that blocks $K_V1.2$ and $K_V1.3$ channels and inhibits T cell activation, a property that could be exploited to develop new treatments for neuroinflammatory diseases and autoimmune disorders.

$K_V1.3$ channels are expressed in peripheral immune cells and are implicated in T cell activation, when an efflux of K^+ is required to balance the influx of Ca^{2+} that drives T cell proliferation and cytokine production (2). $K_V1.3$ inhibitors therefore have the potential to suppress the immune system and treat a variety of autoimmune diseases such as multiple sclerosis and rheumatoid arthritis. $K_V1.3$ channels are also expressed in brain-resident immune cells called microglia, and are therefore also considered potential targets for neuroinflammatory disorders like Parkinson's disease.

"My lab's long-term goal is to find high-affinity, selective inhibitors of $K_V1.3$ channels," explains Gyorgy Panyi of the University of Debrecen in Hungary. Such inhibitors might be found in scorpion venom: Researchers have so far identified nearly 200 scorpion toxins that target K_V channels (3). This includes the α-KTx family of toxic peptides that are typically 30–40 amino acids in length, form an α/β scaffold structure stabilized by 3–4 disulfide bridges, and contain a functional lysine–tyrosine dyad that is crucial for blocking the channel pore and determining the toxin's selectivity.

Panyi and colleagues, including Muhammad Umair Naseem, a graduate student who received a Stipendium Hungaricum Scholarship from the Tempus Public Foundation, set out to characterize a novel peptide isolated from the Columbian scorpion *Centruroides margaritatus* by Lourival Possani's group at the Universidad Nacional Autónoma de México (4). Phylogenetic analysis of this peptide, named Cm28, shows that it clusters with the α-KTx family, and



Muhammad Umair Naseem (seated), Gyorgy Panyi (standing), and colleagues identify a novel scorpion toxin, Cm28, that has a unique primary structure and selectively inhibits $K_V1.3$ channels with high affinity. Electrophysiology (center) shows that Cm28 inhibits $K_V1.3$ currents in human T cells, and, accordingly, flow cytometry (right) shows that it reduces expression of the early activation marker CD40L upon T cell stimulation.

structural modeling suggests that it adopts a similar α/β structure with three disulfide bonds (1). "However, Cm28's primary structure is unique," says Panyi. "It is only 27 amino acids in length, and it lacks the functional dyad typical of α-KTxs."

Cm28 may therefore be the first member of a new subfamily of α-KTxs. Naseem et al. (1) found that it reversibly inhibits $K_V1.2$ and $K_V1.3$ channels with high affinity, showing K_d values of 0.96 and 1.3 nM, respectively.

The peptide shows an ~400-fold lower affinity for $K_V1.1$ channels, and has no effect on a variety of other K^+, Na^+, and H^+ channels at a concentration of 150 nM.

Most toxins known to inhibit K_V channels do so by either blocking the channel pore or by binding to the voltage-sensing domain and modulating channel gating. The researchers determined that Cm28 has no effect on the voltage dependence of channel activation, suggesting that it isn't a gating modifier. Instead, and despite its lack of the typical lysine–tyrosine dyad, Cm28 likely functions as a pore blocker, since the toxin's binding kinetics were consistent with a simple, bimolecular interaction between the peptide and $K_V1.2$/$K_V1.3$ channels.

In keeping with $K_V1.3$'s role in T cells, Naseem et al. found that Cm28 inhibited the activation of human effector memory T lymphocytes. Without affecting cell viability, Cm28 reduced the expression of two key early activation markers following T cell stimulation. This suggests that the toxin could form the basis of new treatments for autoimmune diseases. However, Panyi cautions, much work remains to be done, beginning with the production of functional recombinant Cm28 to supplement the meager amounts of toxin that can be purified from scorpion venom.

"Then, we can determine how Cm28 binds and inhibits $K_V1.3$ channels," Panyi says. "After that, we can engineer the toxin to improve its channel selectivity and pharmacokinetics and begin testing it in animal models."

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Superfast fish show superfast coupling

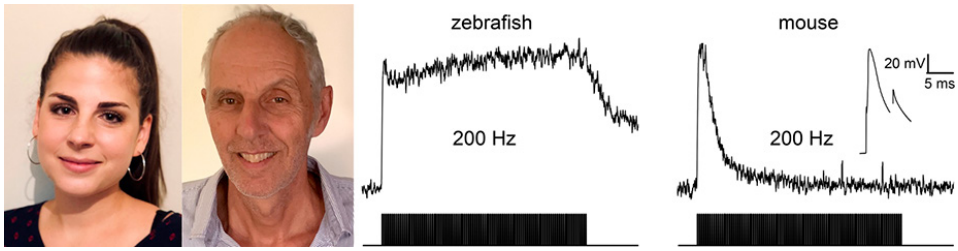
Adult zebrafish skeletal muscle fibers display the fastest kinetics of excitation–contraction coupling ever measured in vertebrate locomotor muscles

The nervous excitation of vertebrate skeletal muscle fibers is coupled to muscle contraction by a series of events in which action potentials propagate along the muscle membranes and trigger conformational changes in voltage-sensitive dihydropyridine receptors (DHPRs) that, via a direct interaction with ryanodine receptors, cause Ca^{2+} to be released from the sarcoplasmic reticulum. To date, the fastest excitation–contraction (EC) coupling kinetics ever recorded have been in the muscle fibers of male toadfish swim bladders, which, rather than generating movement, produce the sound of the animal's mating call (1). However, Idoux et al. (2) describe similarly rapid EC coupling in the locomotor muscles of adult zebrafish.

Zebrafish have a strong startle response that allows them to escape predators and catch prey. This rapid swimming activity is driven by fast muscle fibers that display superfast contraction kinetics, generating fused, tetanic contractions at extremely high frequencies of excitation not seen in mammalian skeletal muscles (3). These superfast kinetics depend, in part, on the properties of contractile proteins in the zebrafish muscle sarcomeres. But they likely also require superfast EC coupling kinetics. "However, although EC coupling has been measured in zebrafish fast skeletal muscle cells, there has been no attempt to compare the kinetics with mammalian fast fibers," explains Bruno Allard, whose research group at the Université Claude Bernard Lyon 1 studies adult zebrafish as a model for human muscular disease.

Allard and colleagues, including first author Romane Idoux, isolated fast muscle fibers from adult zebrafish and mice, and performed a series of voltage- and current- clamp experiments combined with intra- cellular Ca^{2+} measurements to monitor the different steps of EC coupling (2).

First, the researchers compared the action potentials generated in these fibers and found that zebrafish action potentials have a smaller amplitude and repolarize much faster than those seen in mouse muscle fibers. Allard and colleagues speculate that this rapid repolarization, which allows zebrafish muscles to be stimulated at frequencies of almost 200 Hz, could be driven by the Ca^{2+}-activated chloride channel ANO1, which is absent from mammalian muscle but has been shown to accelerate action potentials in zebrafish larval muscle cells (4).



Romane Idoux (left), Bruno Allard (center), and colleagues demonstrate the superfast kinetics of EC coupling in adult zebrafish skeletal muscle fibers that allow the muscles to contract when excited at extremely high frequencies, powering the animal's rapid startle response. When stimulated at 200 Hz for 0.5 s, zebrafish fibers form a fused and sustained Ca^{2+} transient (left trace), whereas in a mouse fiber excited at the same rate (right trace), the initial Ca^{2+} transient quickly returns to baseline because the second stimulation of the train fails to trigger an action potential (inset).

Idoux et al. (2) saw similarly rapid kinetics when they measured the intra- membrane charge movements associated with DHPR activation. This voltage- dependent step occurred up to four times faster in zebrafish than it does in mice, possibly due to species-specific differences in DHPR and its four voltage-sensing domains. Mouse DHPR is a calcium channel whose opening is controlled by one, slowly activating voltage-sensing domain (5). Zebrafish DHPR, in contrast, does not conduct calcium, potentially allowing all four of its voltage-sensing domains to control the rapid activation of ryanodine receptors.

Finally, Idoux et al. (2) measured the intracellular Ca^{2+} transients arising from ryanodine receptor activation. These, too, showed superfast kinetics in zebrafish muscle fibers, with decay rates much faster than those seen in mice, likely due to increased density of a high affinity intracellular Ca^{2+} buffer, probably parvalbumin, as shown by a Ca^{2+} distribution model simulating Ca^{2+} transients built by the authors.

Together, these superfast kinetics of EC coupling allowed adult zebrafish muscle fibers to form fused and sustained Ca^{2+} transients at excitation frequencies of ~200 Hz, similar to the high fusion frequencies previously observed in toadfish swim bladder muscles (1). In contrast, with their slower decay rates, Ca^{2+} transients in mouse muscle fibers fuse at much lower excitation frequencies, and cannot be sustained at 200 Hz as the slow kinetics of action potential repolarization cause the muscle membranes to become refractory.

"To our knowledge, our study is the first demonstration of superfast EC coupling properties in vertebrate skeletal muscles involved in locomotion," Allard says. "The accelerated kinetics of each EC coupling step enable the superfast contraction kinetics of zebrafish fast muscle fibers that are known to be involved in the animal's startle response."

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Kv1 channel inactivation: Slow and slower

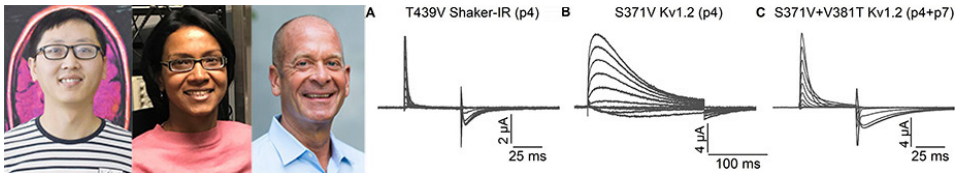
Kv1 channels share a common mechanism of slow inactivation, but some family members are less prone to inactivate than others

Voltage-activated potassium (Kv) channels repolarize neurons and other excitable cells following membrane depolarization. Sustained depolarization, however, inactivates Kv channels in two kinetically and mechanistically distinct ways: a rapid form of inactivation involving blockade of the internal pore, and a slower inactivation process linked to structural changes in the channel's selectivity filter. These inactivation processes are crucial for shaping action potentials and regulating neuronal firing frequency. Wu et al. (1) reveal that members of the Kv1 subfamily have different propensities to undergo slow inactivation, but they likely all do so via an identical structural rearrangement.

Initially described in the *Drosophila* Kv channel Shaker (2), slow inactivation has been shown to depend on a number of amino acids located in the selectivity filter in the external region of the pore. Based on observations of the bacterial potassium channel KcsA, the selectivity filter was initially proposed to collapse during slow inactivation. Earlier this year, however, Kenton Swartz and colleagues at the NIH obtained structural data indicating that the selectivity filter of Shaker actually dilates and inactivates the channel by blocking two potassium-binding sites in the outer pore (3). A recent crystal structure suggests that the selectivity filter of the mammalian Kv1.2 channel undergoes a similar dilation during slow inactivation (4).

A series of conserved amino acids around the selectivity filter form hydrogen bond networks that stabilize the conducting state of the channel (5), and their rearrangement underlies filter dilation (2, 3). Mutating these residues can either speed up or slow down slow inactivation, but some of these mutations have been reported to have different effects on Shaker and Kv1.2 channels (6). "We wanted to do a systematic comparison of how mutations influence slow inactivation in Kv1.2 and Shaker to get a sense of whether the two channels use similar mechanisms," says Swartz.

Swartz and colleagues Xiaosa Wu and Kanchan Gupta therefore generated equivalent mutations in Shaker and Kv1.2 and measured their effects on channel inactivation (1). Several individual point mutations in Shaker accelerate slow inactivation, in some cases making the process so fast that the channels are effectively nonconducting (7). Wu et al. (1) consistently observed that the equivalent mutations in Kv1.2 have less dramatic phenotypes, only slightly accelerating slow inactivation or having no effect at all.



Xiaosa Wu, Kanchan Gupta, and Kenton Swartz (left to right) reveal that members of the Kv1 channel family have different propensities to slow inactivate, but use the same mechanism of selectivity filter dilation. The T439V mutation in the Shaker channel greatly accelerates slow inactivation (A) whereas the equivalent mutation in Kv1.2, S371V, has less of an effect (B). This difference is erased, however, when S371V is combined with a second mutation in a critical residue near the selectivity filter, V381T (C).

The researchers found that these differences depend on one key residue near the selectivity filter, which is a threonine (T449) in Shaker and a valine (V381) in Kv1.2. Mutations in Kv1.2 accelerated slow inactivation to the same extent as the equivalent mutations in Shaker when they were combined with a V381T mutation to make the Kv1.2 channel identical to Shaker at this position, extending a similar observation by others (6).

This suggests that due, in part, to the presence of valine at this critical position, Kv1.2 channels have a lower propensity than Shaker channels to slow inactivate, but the inactivation mechanism involves a similar dilation of the selectivity filter in both channels. "It's a good example of how subtle differences between two channels can make the impact of mutations look very different and lead people to think they're looking at distinct mechanisms when, in fact, they may be similar," Swartz says.

Further work will be required to identify some of the other differences that make Kv1.2 channels less prone to slow inactivation. Swartz and colleagues are also interested in studying other Kv channels, such as Kv2.1, that may, indeed, slow inactivate through a different mechanism.

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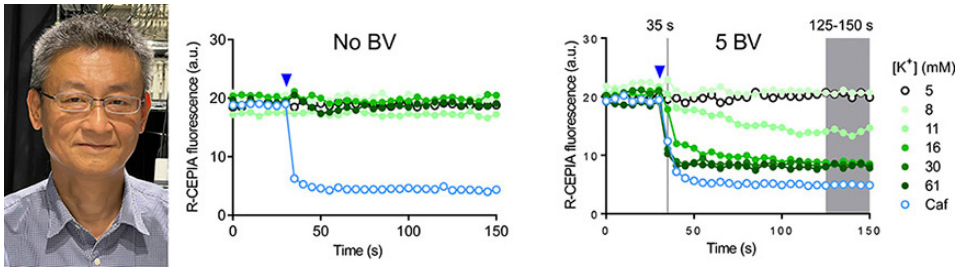
Reconstituting depolarization-induced calcium release

Researchers develop experimental platform that could be used to evaluate mutations and screen drugs for skeletal muscle diseases

Skeletal muscle contraction is initiated by the release of Ca^{2+} from the sarco- plasmic reticulum upon depolarization of the muscle cell plasma membrane. This depolarization-induced Ca^{2+} release (DICR) is mediated by the type I ryanodine receptor (RyR1), a Ca^{2+} release channel in the sarcoplasmic reticulum that is activated by the voltage-sensitive Cav1.1 subunit of the dihydropyridine receptor. A rapidly expanding number of mutations in the genes encoding the DICR machinery have been linked to skeletal muscle diseases such as congenital myopathy and malignant hyperthermia. But evaluation of these mutations, and the development of drugs to correct defects in DICR, has been hampered by the difficulties associated with culturing and manipulating skeletal muscle cells. However, Murayama et al. reconstituted DICR in human embryonic kidney cells, providing a platform to validate mutations and screen drugs in a multiwell format (1).

Working with skeletal muscle cells can be expensive and time consuming but, in 2017, Perni et al. (2) managed to reconstitute DICR in non-muscle cells by patch-clamping tsA201 cells recombinantly expressing RyR1, Cav1.1, and three other proteins required for DICR (the β1a subunit of the dihydropyridine receptor, the adaptor protein Stac3, and the membrane-tethering protein junctophilin-2). "Inspired by their work, we started this study to improve the platform so that it can be applied to multiwell plates," says Takashi Murayama of the Juntendo University School of Medicine in Tokyo.

Murayama and colleagues had previously generated a human embryonic kidney cell line stably expressing both RyR1 and a fluorescent ER Ca^{2+} reporter called R-CEPIA1er (3). To reconstitute DICR in these cells, Murayama et al. infected them with baculoviruses carrying the genes encoding Cav1.1, β1a, Stac3, and junctophilin-2, achieving widespread expression of the DICR machinery. The researchers also used baculovirus to introduce the inward-rectifying potassium channel Kir2.1, hyperpolarizing the cells so that RyR1 is inactive under resting conditions.



Takashi Murayama and colleagues reconstitute DICR in human embryonic kidney cells by using baculovirus to transduce the cells with multiple components of the DICR machinery. Chemical depolarization with high $[K^+]$ solutions elicits no change in ER Ca^{2+} levels in control cells (left) but induces Ca^{2+} release in baculovirus-infected cells (right). The novel platform can be used in multiwell formats and is therefore suitable for validating mutations and drug screening for skeletal muscle diseases.

"All the cells can then be simultaneously stimulated by chemical depolarization with a high $[K^+]$ solution, which provides signals high enough to be measured using a microplate reader," Murayama explains. Crucially, the R-CEPIA1er reporter monitors the depolarization-induced loss of Ca^{2+} from the ER, rather than the depolarization-induced increase in cytoplasmic Ca^{2+}, avoiding potential signal contamination arising from the influx of extracellular Ca^{2+}.

After establishing that the platform faithfully reconstitutes DICR, Murayama et al. used it to evaluate several disease-causing mutations in Cav1.1. The malignant hyperthermia-associated mutation R1086H, for example, shifted the voltage- dependence of DICR in kidney cells to more negative potentials, consistent with previous observations in myotubes (4). This suggests that the platform can be used to validate other, novel mutations in DICR components.

Murayama and colleagues also tested several DICR-modulating drugs in their platform, confirming that the RyR1 inhibitors dantrolene and Cpd1 impede DICR while the twitch potentiator perchlorate sensitizes the modified kidney cells to DICR.

The researchers hope to further improve the platform by introducing additional proteins that, though not essential for DICR, are known to regulate its activity. Then, they hope to use the technology to validate novel disease-linked mutations and screen for drugs that modulate DICR. "Since the procedure is simple and reproducible, we hope that the reconstituted DICR platform will be widely used for the diagnosis and treatment of muscle diseases," Murayama says.

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